K954570

APR 30 1996

510(k) SUMMARY

SUBMITTER: Ortho Diagnostic Systems Inc
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DEVICE NAME:

ORTHO™ ImmunoCount Flow Cytometry System

ORTHO ImmunoCount Flow Cytometry System includes:

ORTHO CytoronAbsolute™ Laser Flow Cytometer (optional AutoBioSampler)

ORTHO ImmunoCount II Software

ORTHO TRIO Monoclonal Antibodies (Murine)

ORTHO TRIO Control (FITC/PE/CyP) Monoclonal Antibodies (Murine)

ORTHO TRIO CD4/CD8/CD3 (OKT™4A/OKT8/OKT3) (FITC/PE/CyP) Monoclonal Antibodies (Murine)

ORTHO TRIO CD16/CD19/CD3 (NK/OKB™19A/OKT3) (FITC/PE/CyP) Monoclonal Antibodies (Murine)

Ortho-Count Calibration Kit
(currently under review, Ref. No. K-935720)

PREDICATE:

FACScan™ Flow Cytometer
Simulset Software
Simultest™ reagents K93292

Simultest Control IgG_1/IgG_{2a} (IgG_1 FITC/IgG_{2a}PE)

Simultest LeucoGATE™ (CD45/CD14[Anti-HLe 1/Leu™M3])

Simultest CD3/CD4 (Leu-4/3a)

Simultest CD3/CD8 (Leu-4/Leu-2a)

Simultest CD3/CD16+CD56 (Leu-4/11c+19)

Simultest T and B Cell Test CD3/CD19 (Leu4/Leu12)

Hematology Analyzers

Coulter T 540	K896873
Coulter JT2	K874383
AGROS	K895127

DATE: September 29, 1995

DEVICE DESCRIPTION:

ORTHO ImmunoCount Flow Cytometry System consists of ORTHO CytoronAbsolute Laser Flow Cytometer, ImmunoCount II Software, ORTHO TRIO Monoclonal Antibodies for lymphocyte immunophenotyping, and Ortho-Count Calibration Kit for calibration and verification of system calibration.

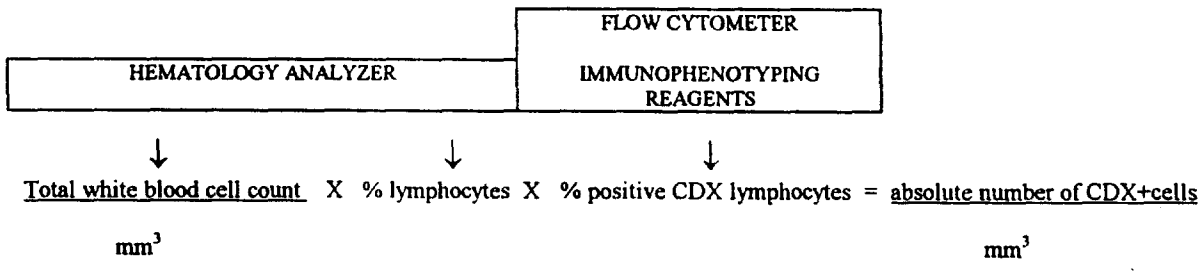
INTENDED USE:

ORTHO ImmunoCount Flow Cytometry System is intended to be used for lymphocyte immunophenotyping. Results may be reported as either percent positive cells, or as absolute counts of lymphocytes and lymphocyte subsets.

TECHNOLOGICAL CHARACTERISTICS:

ORTHO ImmunoCount Flow Cytometry System is substantially equivalent to the combination of flow cytometer, lymphocyte immunophenotyping reagents, and hematology analyzer when used for determination of percentages and absolute counts of lymphocytes and lymphocyte subsets.

The traditional method for determination of absolute counts of lymphocyte subsets combines lymphocyte immunophenotyping reagents and a flow cytometer for determination of lymphocyte subset percentages, with a hematology analyzer for determination of total lymphocyte count, to obtain absolute counts of lymphocyte subsets.



PERFORMANCE DATA:

ORTHO ImmunoCount Flow Cytometry System was compared to the traditional method for determining absolute counts of lymphocytes and lymphocyte subsets in a clinical study conducted at four laboratories. The instruments and reagents used in the clinical evaluation of the ORTHO ImmunoCount Flow Cytometry System are:

FACScan™ Flow Cytometer with Simulset Software
- Becton Dickinson Immunocytometry Systems

Simultest™ Monoclonal Antibody Immunophenotyping Reagents
-Becton Dickinson Immunocytometery Systems

Simultest Control IgG_1/IgG_{2a} (IgG_1 FITC/IgG_{2a}PE)
Simultest LeucoGATE™ (CD45/CD14[Anti-HLe-1/Leu™-M3])
Simultest CD3/CD4 (Leu-4/3a)
Simultest CD3/CD8 (Leu-4/Leu-2a)
Simultest CD3/CD16+CD56 (Leu-4/11c+19)
Simultest T and B Cell Test - CD3/CD19 (Leu4/Leu12)

Coulter® JT2 Automated Hematology Analyzer
-Coulter Electronics

Coulter T Series Automated Hematology Analyzer
-Coulter Electronics

Argos Hematology Analyzer
-ABX France

Blood specimens were collected from 200 apparently healthy donors. Normal donors were defined as any person who was not clinically diagnosed with an immunodeficiency disease or hematological malignancy. The donors were male (44.5%) and female (55.5%) with an age range of 21 years to 62 years. Three geographically distinct clinical sites in the United States were used to determine absolute lymphocyte counts and four geographically distinct clinical sites in the United States were used to determine the percent positive stained cells. A total of 200 normal whole blood specimens were stained with ORTHO TRIO Monoclonal Antibodies and analyzed in the ORTHO ImmunoCount Flow Cytometry System for percent positive stained cell determination. A total of 151 normal whole blood specimens were stained with ORTHO TRIO Monoclonal Antibodies and analyzed in the ORTHO ImmunoCount Flow Cytometry System for absolute lymphocyte count determination.

TABLE A

IMMUNOCOUNT SYSTEM LYMPHOCYTE SUBSET PERCENT POSITIVE					
NORMAL DONORS N = 200					
ImmunoCount	Mean%	Range%	Predicate	Mean%	Range%
$CD3^+CD4^+$	45.5	20 - 68	$CD3^+CD4^+$	45.2	20 - 65
$CD3^+$ (4/8/3)	74.5	36 - 101	$CD3^+$ (3/4)	73.2	29 - 91
$CD3^+CD8^+$	24.5	9 - 48	$CD3^+CD8^+$	26.9	9 - 55
$CD3^+$ (4/8/3)	74.5	36 - 101	$CD3^+$ (3/8)	73.5	31 - 90
$CD16^+CD3^-$	11.6	1 - 58	$CD(16\&56)^+ CD3^-$	13.1	2 - 65
$CD3^+$ (16/19/3)	75.1	39 - 89	$CD3^+$ (3/16&56)	73.6	31 - 91
$CD19^+$	13.3	3 - 51	$CD19^+$	13.3	3 - 28
$CD3^+$ (16/19/3)	75.1	39 - 89	$CD3^+$ (3/19)	73.3	30 - 89
Mean $CD3^+$	74.8	38 - 95	Mean $CD3^+$	73.4	30 - 90

IMMUNOCOUNT SYSTEM LYMPHOCYTE SUBSET ABSOLUTE COUNTS					
NORMAL DONORS N = 151					
ImmunoCount	Mean (cells/µl)	Range (cells/µl)	Predicate	Mean (cells/µl)	Range (cells/µl)
$CD3^+CD4^+$	855	257 - 1785	$CD3^+CD4^+$	900	250 - 1940
$CD3^+$ (4/8/3)	1417	477 - 3372	$CD3^+$ (3/4)	1472	480 - 3530
$CD3^+CD8^+$	481	112 - 1588	$CD3^+CD8^+$	551	100 - 1750
$CD3^+$ (4/8/3)	1417	477 - 3372	$CD3^+$ (3/8)	1477	470 - 3500
$CD16^+CD3^-$	221	19 - 1536	$CD(16\&56)^+ CD3^-$	266	60 - 2280
$CD3^+$ (16/19/3)	1428	512 - 3423	$CD3^+$ (3/16&56)	1476	470 - 3530
$CD19^+$	255	35 - 826	$CD19^+$	270	50 - 840
$CD3^+$ (16/19/3)	1428	512 - 3423	$CD3^+$ (3/19)	1469	480 - 3540
Mean $CD3^+$	1423	494 - 3398	Mean $CD3^+$	1473	475 - 3525
ISUM	1905	667 - 4241	lymphocyte	2010	700 - 4159

Blood specimens were collected from 119 HIV-antibody positive donors. HIV-antibody positive donors were defined as any individual positive for the HIV antibody inclusive of AIDS or AIDS Related Complex (ARC) according to the current criteria for these diseases, Morbidity Mortality Weekly Report, Vol. 41/No. 31, May 8, 1992). The donors were male (88.1%) and female (11.9%) with an age range of 3 years to 75 years. Three geographically distinct clinical sites in the United States were used to determine absolute lymphocyte counts and four geographically distinct clinical sites in the United States were used to determine the percent positive stained cells. A total of 119 HIV-antibody positive whole blood specimens were stained with ORTHO TRIO Monoclonal Antibodies and analyzed in the ORTHO ImmunoCount Flow Cytometry System for percent positive stained cell determination. A total of 89 HIV-antibody positive whole blood specimens were stained with ORTHO TRIO Monoclonal Antibodies and analyzed in the ORTHO ImmunoCount Flow Cytometry System for absolute lymphocyte count determination.

TABLE B

IMMUNOCOUNT SYSTEM LYMPHOCYTE SUBSET PERCENT POSITIVE					
HIV ANTIBODY POSITIVE DONORS N = 119					
ImmunoCount	Mean%	Range%	Predicate	Mean%	Range%
$CD3^+CD4^+$	17.7	0 - 55	$CD3^+CD4^+$	17.6	0 - 56
$CD3^+$ (4/8/3)	80.1	18 - 115	$CD3^+$ (3/4)	78.1	24 - 96
$CD3^+CD8^+$	57.3	7 - 89	$CD3^+CD8^+$	58.6	16 - 91
$CD3^+$ (4/8/3)	80.1	18 - 115	$CD3^+$ (3/8)	78.3	21 - 96
$CD16^+CD3^-$	8.8	0 - 45	$CD(16\&56)^+CD3^-$	10.6	1 - 50
$CD3^+$ (16/19/3)	80.9	23 - 95	$CD3^+$ (3/16&56)	78.4	14 - 97
$CD19^+$	10.4	2 - 42	$CD19^+$	10.8	2 - 40
$CD3^+$ (16/19/3)	80.9	23 - 95	$CD3^+$ (3/19)	77.4	22 - 95
Mean $CD3^+$	80.5	21 - 103	Mean $CD3^+$	78.1	20 - 96
IMMUNOCOUNT SYSTEM LYMPHOCYTE SUBSET ABSOLUTE COUNTS					
HIV ANTIBODY POSITIVE DONORS N = 89					
ImmunoCount	Mean (cells/µl)	Range (cells/µl)	Predicate	Mean (cells/µl)	Range (cells/µl)
$CD3^+CD4^+$	260	0 - 1029	$CD3^+CD4^+$	313	0 - 1400
$CD3^+$ (4/8/3)	1078	42 - 2986	$CD3^+$ (3/4)	1262	50 - 3650
$CD3^+CD8^+$	758	39 - 1961	$CD3^+CD8^+$	931	40 - 2810
$CD3^+$ (4/8/3)	1078	42 - 2986	$CD3^+$ (3/8)	1267	40 - 3600
$CD16^+CD3^-$	93	5 - 572	$CD(16\&56)^+CD3^-$	144	20 - 720
$CD3^+$ (16/19/3)	1079	34 - 2927	$CD3^+$ (3/16&56)	1267	30 - 3650
$CD19^+$	131	13 - 554	$CD19^+$	170	20 - 870
$CD3^+$ (16/19/3)	1079	34 - 2927	$CD3^+$ (3/19)	1248	40 - 3650
Mean $CD3^+$	1078	38 - 2957	Mean $CD3^+$	1261	40 - 3638
ISUM	1303	118 - 3691	lymphocyte	1568	200 - 4600

Linear regression analyses of absolute lymphocyte counts and percent positive cells obtained using the ORTHO ImmunoCount Flow Cytometry System and the traditional (predicate) method are presented below.

TABLE C

NORMAL N = 200
AND
HIV ANTIBODY POSITIVE N = 119

ImmunoCount	Predicate	Graph	R	Slope	Intercept
$CD4^+$ (4/8/3)	$CD4^+$ (3/4)	3a	1.00	1.00	0
$CD3^+$ (4/8/3)	$CD3^+$ (3/4)	3b	0.83	0.87	12
$CD8^+$ (4/8/3)	$CD8^+$ (3/8)	3c	0.98	1.01	-2
$CD3^+$ (4/8/3)	$CD3^+$ (3/8)	3d	0.85	0.90	9
$CD16^+$ $CD3^-$ (16/19/3)	$CD16^+$ $CD3^-$ (3/16+65)	3e	0.91	0.90	0
$CD3^+$ (16/19/3)	$CD3^+$ (3/16+56)	3f	0.93	0.88	11
$CD19^+$ (16/19/3)	$CD19^+$ (3/19)	3g	0.88	0.98	0
$CD3^+$ (16/19/3)	$CD3^+$ (3/19)	3h	0.91	0.91	9
Mean $CD3^+$	Mean $CD3^+$	3i	0.91	0.91	9

TABLE D

NORMAL N = 151
AND
HIV ANTIBODY POSITIVE N = 89

ImmunoCount	Predicate	Graph	R	Slope	Intercept
$CD4^+$ (4/8/3)	$CD4^+$ (3/4)	3A	0.94	0.89	25
$CD3^+$ (4/8/3)	$CD3^+$ (3/4)	3B	0.89	0.84	119
$CD8^+$ (4/8/3)	$CD8^+$ (3/8)	3C	0.92	0.78	43
$CD3^+$ (4/8/3)	$CD3^+$ (3/8)	3D	0.89	0.84	112
$CD16^+$ $CD3^-$ (16/19/3)	$CD16^+$ $CD3^-$ (3/16+65)	3E	0.94	0.76	7
$CD3^+$ (16/19/3)	$CD3^+$ (3/16+56)	3F	0.88	0.84	123
$CD19^+$ (16/19/3)	$CD19^+$ (3/19)	3G	0.89	0.86	10
$CD3^+$ (16/19/3)	$CD3^+$ (3/19)	3H	0.89	0.85	124
Mean $CD3^+$	Mean $CD3^+$	3I	0.89	0.84	118
ISUM	Lymphocyte	3J	0.88	0.86	87
All lymphocyte subsets	All lymphocyte subsets	3K	0.96	0.89	3

WITHIN LABORATORY REPRODUCIBILITY

Five normal whole blood specimens were used in a within-laboratory reproducibility study at three independent laboratories. The normal whole blood specimens were stained with ORTHO TRIO Monoclonal Antibodies and run in the ORTHO ImmunoCount Flow Cytometry System. The samples were run diluted (0.5X WBC), concentrated (4X WBC) and undiluted to simulate low, high and normal samples. The leukocyte-depleted blood was prepared using a Sepracell™ unit (Baxter Fenwall). Buffy coat preparations were prepared from the donor samples, counted and adjusted to a 4X concentration. Serial dilutions were made from the 4X concentrate of each sample's buffy coat and diluting this concentrate with leukocyte depleted whole blood from the same sample. All samples were run in replicates of 10. The coefficients of variations (CV) for normal, concentrated and diluted samples were compared and are contained in Table E. The coefficient of variation was generated from variability between the ten replicates from each of the three laboratories. The low CV values demonstrate strong within-laboratory reproducibility for each lymphocyte subset. The CV values for normal and low counts for CD16+ and CD19+ values were higher due to the low counts for these populations. The higher CVs for CD16+ and CD19+ values do not significantly affect the ImmunoSum (ISUM) CV.

TABLE E
ORTHO IMMUNOCOUNT SYSTEM
WITHIN-LABORATORY REPRODUCIBILITY
COEFFICIENT OF VARIATION OF ABSOLUTE LYMPHOCYTE COUNTS

N(LOW) = 139; N(NORMAL) = 142; N(HIGH) = 146

	LOW	NORMAL	HIGH
ORTHO ImmunoCount System	CV	CV	CV
$CD3^+$ (4/8/3)	5.2	4.9	4.3
$CD3^+CD4^+$	6.2	5.2	4.5
$CD3^+CD8^+$	6.4	6.4	4.5
$CD16^+CD3^-$	16.3	10.1	6.0
$CD3^+$ (16/19/3)	5.0	4.9	4.3
$CD19^+$	12.0	10.0	5.7
ISUM Lymph Count	5.0	4.9	4.2

BETWEEN LABORATORY REPRODUCIBILITY

Five normal whole blood specimens were compared between three independent laboratories. The whole blood specimens were stained with ORTHO TRIO Monoclonal Antibodies and analyzed in the ORTHO ImmunoCount Flow Cytometry System. Low, normal and high count preparations were made according to the method described in the Within-Laboratory Reproducibility section. All samples were run in replicates of ten. The data collected from all laboratories are shown in Table F. The coefficient of variation was generated by comparing the three laboratories absolute lymphocyte subset means from the 10 replicates. The low CV values demonstrate acceptable between-laboratory reproducibility at all three laboratories. The CV values for normal and low counts for CD16+ and CD19+ values were higher due to the low counts for these populations. The higher CVs for CD16+ and CD19+ values do not significantly affect the ImmunoSum (ISUM) CV.

TABLE F
ORTHO IMMUNOCOUNT SYSTEM
BETWEEN-LABORATORY REPRODUCIBILITY
COEFFICIENT OF VARIATION OF ABSOLUTE LYMPHOCYTE COUNTS

LOW COUNT PREPARATION : N =139				
ORTHO ImmunoCount System	Lab 1 Mean Cells/µL	Lab 2 Mean Cells/µL	Lab 3 Mean Cells/µL	Between-site CV
$CD3^+$(4/8/3)	750	740	736	0.9
$CD3^+CD4^+$	481	480	473	0.9
$CD3^+CD8^+$	231	221	224	2.2
$CD16^+ CD3^-$	146	122	160	13.1
$CD3^+$(16/19/3)	754	748	740	0.9
$CD19^+$	116	98	121	10.3
ISUM Lymph count	1011	963	1009	2.6
NORMAL COUNT PREPARATION: N = 142				
ORTHO ImmunoCount System	Lab 1 Mean Cells/µL	Lab 2 Mean Cells/µL	Lab 3 Mean Cells/µL	Between-site CV
$CD3^+$(4/8/3)	1494	1468	1445	1.6
$CD3^+CD4^+$	958	953	933	1.3
$CD3^+CD8^+$	456	434	437	2.6
$CD16^+ CD3^-$	363	290	309	11.5
$CD3^+$(16/19/3)	1502	1515	1489	0.8
$CD19^+$	221	212	250	8.5
ISUM Lymph count	2067	2002	2028	1.6
HIGH COUNT PREPARATION: N =146				
ORTHO ImmunoCount System	Lab 1 Mean Cells/µL	Lab 2 Mean Cells/µL	Lab 3 Mean Cells/µL	Between-site CV
$CD3^+$(4/8/3)	5961	5718	5663	2.7
$CD3^+CD4^+$	3786	3687	3667	1.7
$CD3^+CD8^+$	1835	1693	1683	4.8
$CD16^+ CD3^-$	1548	1334	1347	8.4
$CD3^+$(16/19/3)	5990	5929	5737	2.2
$CD19^+$	962	844	999	8.3
ISUM Lymph count	8417	8028	7959	3.0

SPECIMEN AGE CRITERIA

A total of 64 whole blood specimens, normal (34 donors) and HIV-antibody positive (30 donors), were tested at three external clinical sites over an extended time period. The samples were collected in EDTA anticoagulant tubes. Samples were prepared and tested within 24 hours, 48 hours and 72 hours after collection. The absolute counts for each ORTHO TRIO Monclonal Antibody, run in the ORTHO ImmunoCount Flow Cytometry System, are compared in below. The data demonstrates that samples processed within 24 to 72 hours of collection produce comparable results. CI refers to confidence interval of the mean slope.

TABLE G

ORTHO IMMUNOCOUNT SYSTEM
ABSOLUTE LYMPHOCYTE COUNTS OVER A 72-HOUR TIME PERIOD
NORMAL DONORS N=34; HIV-ANTIBODY POSITIVE DONORS N=30

ORTHO ImmunoCount System	Mean Count 24 Hours (Cells/μL)	Mean Count 48 Hours (Cells/μL)	Mean Count 72 Hours (Cells/μL)	Mean Slope	CI
$CD3^+$(4/8/3)	1367	1388	1362	-1.5	2.3
$CD3^+CD4^+$	615	631	619	-0.4	1.0
$CD3^+CD8^+$	664	665	653	-1.2	1.3
$CD16^+CD3^-$	188	199	249	0.8	1.4
$CD3^+$ (16/19/3)	1360	1356	1338	-3.6	2.4
$CD19^+$	207	206	192	-0.0	0.7
ISUM Lymph Count	1755	1761	1779	-2.9	2.6

LINEARITY

Four normal whole blood specimens were used in a linearity study at Ortho Diagnostic Systems. The whole blood specimens were stained with ORTHO TRIO Monoclonal Antibodies and run in the ORTHO ImmunoCount Flow Cytometry System. The samples were run diluted (0.5X, 0.25X, 0.125X, 0.065X), concentrated (5X, 4X, 2X) and undiluted (1X) to yield low, high, and normal cell counts. The leukocyte depleted blood was prepared using a Sepracell™ unit (Baxter Fenwall.) Buffy coat preparations were prepared from the donor sample, counted and diluted to a 5X concentration. Serial dilutions were made fromt he 5X concentrate of each samples buffy coat and diluting this concentrate with leukocyte depleted whole blood from the same unit. Table H shows the correlation coefficient of the linear regression analysis of absolute counts for each marker and ISUM.

TABLE H

ORTHO IMMUNOCOUNT SYSTEM LINEAR RANGE OF ABSOLUTE COUNTS NORMAL DONORS N=4		
ORTHO ImmunoCount System	R	Range Cells/μL
$CD3^+$(4/8/3)	1.00	75 - 9829
$CD3^+CD4^+$	1.00	0 - 6300
$CD3^+CD8^+$	1.00	0 - 2952
$CD16^+CD3^-$	0.99	16 - 1796
$CD3^+$ (16/19/3)	1.00	59 - 10390
$CD19^+$	1.00	19 - 2507
ISUM Lymph Count	1.00	95 - 14694

CONCLUSION

- The performance of the ImmunoCount Flow Cytometry System was equivalent to the predicate method for the determination of absolute lymphocyte counts and lymphocyte subset percentages and absolute counts CD3+, CD3+CD4+, CD3+CD8+, CD16+CD3-, and CD19+.

- ORTHO ImmunoCount Flow Cytometry System demonstrated excellent within and between laboratory reproducibility for low, normal, and high absolute counts for total lymphocytes and lymphocyte subsets.

- ORTHO ImmunoCount Flow Cytometry System, when used to assay samples at extended time points after collection (48 through 96 hours after collection), showed that samples assays up to 72 hours after collection produced equivalent absolute counts for total lymphocytes and for each of the lymphocyte subsets CD3+, CD3+CD4+, CD3+CD8+, CD16+CD3-, and CD19+

- ORTHO ImmunoCount Flow Cytometry System demonstrated excellent linearity when compared against dilution over a wide range of absolute counts for each lymphocyte subset and ISUM.